SCIENCE

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RECENT STUDIES ON THE BIOLOGICAL EFFECTS OF RADIOACTIVITY[1]

X-RAYS were discovered in 1895 and the first of the publications which placed Madame Curie, the discoverer of radium, in the position of foremost woman of science, appeared in 1898. The application of these results to biology, a matter of great importance, was brought about through accident. A knowledge of the physical properties of radio-active substances would lead one to expect that the physiological action would be acute, and that fact was accidentally proven to be true.

Becquerel carried a small tube of an impure radium preparation in his vest pocket for six hours. A few days later he observed a reddening of the epidermis of the abdomen opposite the location of the pocket in which he had placed the radium compound. It was not long before the inflammation became pronounced, and an ulcer developed which required several months for the healing.[2]

Giesel exposed the inner portion of his arm, for two hours, to 0.27 gram of a radium preparation, enclosed with a double celluloid capsule. After two or three weeks the skin reddened, blisters formed and the epidermis peeled just as with a burn. The growth of hair was also destroyed and did not come out anew, although a smooth white skin reformed.

Madame Curie had learned very early in her studies that radiation affects tissues, for she says in her thesis, "The action of radium upon the skin can take place across metal screens, but with weakened effect."

Thus early began the application of a

[1] Contribution from the Zoological Laboratory of the University of Texas, No. 125.

[2] From Baskerville, "Radium and Radioactive Substances," Williams, Brown and Earle, Philadelphia, 1905.

method which has now an established place in therapeutics, in the treatment of skin diseases, warts, tumors, cancers, etc. An imposing list of literature from the clinical standpoint bears witness to the practical importance of radioactivity, and many contributions show the interest of medical men. Now all physicians must be familiar with the results of work in this line, and, in addition, every city boasts its experts and specialists in radiography and radiotherapy.

Thus far, however, clinicians have been chiefly concerned with the formulation of effective methods of treatment, and it has been left for the biologists rather than the physicians to analyze the effects of radioactivity upon living matter.

The attack on the problem of the effect of radioactivity on tissues and organisms and the use of radioactivity as an experimental means of studying questions of more fundamental biological importance was at first insignificant. Even yet our knowledge of the effects is very meager; and to our ignorance of the deeper-lying basic principles which govern the action of radium and of X-rays is due the uncertainty with which the extension of the methods to new fields and new problems is viewed by many medical men. The analysis of the effects has been taken up only recently and progress in the investigation has not been rapid, with the result that very little constructive work has been done.

In studying the effects of radioactivity, both radium and X-rays have been used as a means of experiment, and the literature of both may be considered together on the basis of the current working hypothesis that the effects of both are comparable, especially in the case of the gamma rays of radium. Radium rays are of three kinds, alpha, beta and gamma; of these the gamma rays are the most penetrating, and to them are probably due most of the effects on living forms. From comparative studies of the physicists it is well known that the gamma rays of radium are quite similar in many particulars to the X-rays, and it is stated by Rutherford that they are, in fact, the more penetrating X-rays. Some recent experiments seemed to indicate that the effect of the other rays is by no means negligible, for with the preparation used, when the rays were deflected by a magnet the effect of the beta rays was stronger than that of either of the others. Even in this case, however, the effects seem to differ in degree rather than in the kind of their action, and the results are not in conflict with the hypothesis which is now serving as a working basis for experiment.

In general, it may be said that when living cells are exposed to action of radioactivity, the vital functions are retarded or depressed and a permanent injury may result; this depends on three factors, the strength of the radiating substance, the duration of the exposure, and the distance of the object from the source of the radiation. When the intensity of the radiation is great, owing to exposure at short range to a strong preparation (or strong current in the case of X-rays) for a long time, the effects are much more injurious than when the intensity is less. Indeed, numerous cases have been reported where a qualitative difference results from a slight radiation as contrasted with one of great intensity, for frequently stimuli which will retard growth if of high degree, will be found to accelerate it if weak enough. Exposure to rays of great intensity has been shown to retard or stop growth, differentiation and regeneration, and to interfere with the processes of cell division, sometimes causing degenerative changes to take place in the nuclei, and in one case at least to induce amitosis where indirect cell division had normally been the method of multiplication.

In rapidly growing tissue, such exposure will cause a decrease in the rate of division as well as interfering with its regularity. On the other hand, an exposure of short duration and of slight intensity will in some cases stimulate growth, and accelerate regeneration, and may perhaps cause an increase in the rate of cell division.

The literature[3] on radioactivity and its biological effects is voluminous, but there are only a small number of papers dealing with the question from a biological point of view. There were a very few early papers on the effect on growth and cell division; they, however, were pioneers in this field and their results are not far reaching. Most of the work done at that time, and indeed the majority of all work on the problem, has sought to use radioactivity for the study and solution of questions which were purely medical. Of the early experiments those of Perthes,[4] of Schwarz[5] and of Schaper[6] have perhaps had the most bearing on the later development of the problem. These early investigations were done about 1903–05.

[3] No attempt is here made to offer a comprehensive bibliography on this subject, for such lists are available in Gager, C. Stuart, "Effects of the Rays of Radium on Plants" (*Mem. N. Y. Bot. Garden*, IV., 1908); in Warthin, A. S., "An Experimental Study of the Effects of Röntgen Rays on the Blood-forming Organs with Special Reference to the Treatment of Leukæmia" (*International Clinics*, Vol. 47, 1906); in the publications of Bardeen, of the Hertwigs and others. Only references are here included which bear directly on our present theories for the interpretation of the observed effects.

[4] Perthes, G., "Versuche ueber den Einfluss der Röntgen- und Radiumstrahlen auf Zellteilung," *Deutsche Med. Wochenschr. Jahrg.*, Bd. 30, 1904.

[5] Schwarz, G., "Ueber die Wirkung den Radiumstrahlen; eine physiologische chemische Studie am Huhnerei," *Arch. f. Physiol.*, Bd. 100, 1903.

[6] Schaper, A., "Experimentelle Untersuchung ueber den Einfluss der Radiumstrahlen auf Embryonale und regenerative Vorgänge," *Anat. Anz.*, Bd. 25, 1904.

The pioneer stage of the investigations may be considered to end with Gager, who has been the most important botanist to make contributions to this subject. In his monograph he sums up all of the work that has been done and adds many facts from his own experiments. The work of all these men is representative, and may be regarded as showing the state of progress at that time. The results of each made a distinct contribution to our knowledge of the effects of radioactivity, and are here considered in their chronological order because of their bearing on the subsequent development of the subject.

The work of Schwarz is of importance because from his experiments he erected a hypothesis, the lecithin hypothesis, to explain the destructive effects of radioactivity. His hypothesis "was based on the fact that egg yolk is decomposed by exposure to the radium radiations. Although the matter was not chemically determined, it seemed probable that the lecithin was broken up into cholin and tri-methylamine and other end products of lecithin decomposition. Lecithin has been found by many investigators in all cells, especially in egg yolk, spermatozoa, pollen cells, plant spores, growing buds, and in all rapidly growing tissue. If, then, it is destroyed, such cells must necessarily be unfavorably affected" (Packard). According to Schwarz the effect of radiation on chromosomes and other cell organs is an indirect one where it occurs at all, being brought about as a secondary result of the decomposition set up. It is especially at this point that the lecithin hypothesis comes into conflict with that of Hertwig, to be discussed later.

Perthes' observations were among the first from a biological point of view. He exposed *Ascaris* eggs to radium and noted that the cell divisions became slower than in the controls, where no exposure had been

made. The controls gave rise largely to active worms, but radiated eggs developed only into irregular cell-masses or misshapen little worms which were especially irregular at the tail end, the results depending upon whether stronger or weaker intensity of radiation was used. He says that all three elements which normally take part in mitosis were formed. Centrosomes, spindles and fibers were alike all clear in the experiment and in the controls. The chromosomes divided irregularly, and in *Ascaris megalocephala univalens* the characteristic number was doubled, knotty swellings appearing on the chromatin loops instead of the normal arrangement, and in some places the chromosomes were broken up into numerous unequal pieces. The injury to the eggs expressed itself in the slowing down of the development, which gives rise to abnormal individuals. The results with X-rays were entirely analogous to those with radium, delaying cell division and giving rise to abnormal products of development. In both cases, the chief effects of radiation on the cells appeared indirectly, but only after the lapse of a certain period of time. Eggs in the resting and in the dividing condition served equally well for the experiments.

Schaper exposed frog eggs as well as *Triton* embryos to radium for varying lengths of time. He observed an inhibitory effect on cell division, on embryonic differentiation, and on growth. There was also an inhibition of regeneration which was recognized after a longer or shorter period. The latent period usually lasted almost a day, the duration depending on the intensity of the radiation and on the stage of development of the organism. The period of development was always more or less retarded and prolonged. Finally it reached a standstill and then death resulted. He thinks there is some relation between the manner of solution of the yolk and the effect of the radiation. In older larvæ, the living substances were used up and acute degenerative changes in the cells set in.

Gager[7] reviews the literature on this subject up to 1908 and summarizes the state of knowledge at that time in his last paragraph as follows:

> The broadest, and at the same time the most definite generalization warranted by the work so far done is that the rays of radium act as a stimulus to metabolism. If this stimulus ranges between minimum and optimum points, all metabolic activities, whether constructive or destructive, are accelerated, but if the stimulus increases from the optimum toward the maximum point it becomes an over-stimulus, and all metabolic activities are depressed and finally completely inhibited. Beyond a certain point of over-stimulus recovery is impossible, and death results.

His review of the previous investigations he brings together in the following statements:

1. Radium rays have the power to modify the life processes of both plants and animals.
2. Röntgen rays and radium rays produce similar physiological results.
3. Sensitiveness to these rays varies with the species of either plant or animal.
4. Younger, and especially embryonic tissues, are more sensitive than those more mature.
5. With only one or two exceptions, exposure to radium rays has been found to either retard or completely inhibit all cell-activities. The rays may cause irregularities in mitosis.
6. Experimental evidence for or against the existence of a radiotropic response is conflicting.
7. Whatever the immediate, internal change produced in the protoplast may be, the result, with animals as well as with plants, appears to be more or less profoundly modified by the presence of chlorophyll in the cell.
8. Radium rays appear to retard the activity of enzymes.

Since the publication of Gager's results on the effect of radium on plants a large amount of work has been done in the labo-

[7] *Loc. cit.*

ratory of Oskar Hertwig[8] at Berlin. Hertwig himself, his son Günther, and his daughter Paula, and a number of his students have performed an extensive series of experiments over a wide range of forms and have obtained results which are of the greatest significance. In all of their work, radium compounds have been the source of the rays used for experimental purposes. As a strong exposure to radioactivity is always injurious to tissues, and since the development of injured eggs gives rise to malformation and produces monsters of various degrees of deformity, much of the experimental work is teratological in nature. This is interesting from a pathological standpoint, but is perhaps less fundamental than the effects of the radiation on cells (*e. g.*, egg cells) and on their activity. On both these phases of the study the work of the Hertwigs has an important bearing.

The theory which was developed by the early work of the Hertwigs and which has been the working hypothesis upon which their subsequent studies have been made is called by its author a "biological hypothesis." The observation was made in the first cytological studies that centrosomes, spindles and other cell organs with the single exception of the chromatin showed little injury due to the action of the rays. This conclusion was based on evidence from the study of eggs and sperm of sea urchins and of frogs; later the observations have been extended to other forms. It led to the assumption that the effect is a direct one on the chromatin of the radiated cells, not an indirect one as had been postulated by Schwarz, and, further, that the seat of the injury if not exclusively in the chromatin is certainly chiefly there. Due to the fact that a slight radiation of the sperm is sufficient to cause abnormalities in the embryo, it was held that the injured chromatin possesses the property of conveying the injury to the egg cell when it fuses with it and subsequently to the descendants of this cell, for nuclear division provides the mechanism for distributing the injury to all cells of the body. In a sense, therefore, the original injury tends to increase as development proceeds. Hertwig sees in the beta and gamma rays of radium a reagent which affects the nuclear substance of living cells even in the slightest amount. Especially the chromatin is injured in its living properties by the slightest exposure to radio-active rays, and by a greater exposure is so changed that it loses the capacity to grow and to increase in the regular way by mitosis, and undergoes a gradual degeneration into which the cytoplasm is also drawn.

It may be said that this hypothesis has much morphological basis and that it is sufficiently elastic to accord with many of the observed facts; yet it is clear that no real explanation of the phenomena has been offered on this basis, for the problem is simply pushed further back into the cell and it is necessary to make clear how the chromatin is injured and how the injury accumulates with development. It is undoubtedly true also that other substances in the cell than chromatin are injured, although it may not be possible to attribute the irregularities of later development to them, as can be done in the case of the chromatin. A comparison of this hypothesis with the lecithin hypothesis, and criticisms which have been made of each, may be deferred until other facts have been brought out.

Recently Oskar Hertwig[9] has brought together in a brief statement the facts most

[8] A series of papers by O., G., and P. Hertwig, by Oppermann, Fraenkel, and Stachowitz in the *Arch. f. Mikr. Anat.*, Bd. 77 to Bd. 85, 1911–1914.

[9] Hertwig, O., "Versuche an Tritoneiern ueber die Einwirkung bestrahlter Samenfäden auf die tierische Entwicklung," *Arch. f. Mikr. Anat.*, Bd. 82, Abt. II., 1913.

important from his interpretation which have been obtained as the results of the investigations in his laboratory. The following facts are emphasized by him:

1. Fertilized *Ascaris* eggs, which had been radiated several hours showed pathological nuclear division figures in which the chromatin bodies are represented by irregular masses of chromatin granules. They divide slowly and begin at last to degenerate by caryolysis. (Paul Hertwig.)

2. By intensive radiation of several hours' duration, sperm threads of the sea-urchin are so affected that, while they are able to fertilize the egg and to stimulate the egg nucleus into spindle formation, they lose their ability to form normal chromosomes and thus are eliminated from development partially or completely, sooner or later, depending on the degree of the injury. (Günther Hertwig.)

3. An elimination of the sperm nucleus which is derived from intensively radiated sperm threads has been observed during the first and second divisions in eggs of the frog (Paula Hertwig) and of the trout (Oppermann).

4. For the elimination from the development process of the radiated sperm chromatin which has lost its capacity to develop, the fact established beyond doubt for the radiated larvæ of *Triton* speaks convincingly, that the somatic nuclei have only half, or the reduced, number of chromosomes. Since the male chromosome complex fails to take its part in the development, due to the radiation, the somatic nuclei have only the female complex. (Oskar Hertwig.)

5. This fact agrees with the result obtained for frog, toad, *Triton* and trout embryos, that after the maximum radiation of the sperm which are to be used for fertilization, the nuclei of the different cells are strikingly smaller than are those of the controls of the same age, and both their surfaces and their volumes are in ratio to those of the control as 1:2. For the conclusion, that the chromosome number of the nucleus is the haploid, may be drawn from the fact established by numerous experiments that the volumes are reduced almost to half. (Oskar Hertwig, Günther Hertwig, Oppermann.)

6. The results obtained from cytological investigation offer the possibility of explaining a fact highly remarkable from a physiological standpoint and at first glance a very puzzling one, that eggs, which would ordinarily fail to develop from the germinal vesicle stage when fertilized by foreign sperm because of the union between disharmonious idioplasms, are spared from destruction and may develop into larvæ if only the sperm from the different species are radiated intensively before fertilization. The puzzle is solved by the simple reflection that the effect of the union of the disharmonious idioplasms with its disastrous consequences is avoided by the injury to the radiated chromatin, although the sperm thread penetrates into the egg and stimulates development. Although the radiation of the strange sperm has been destructive to the sperm, it has been favorable to the fertilized egg, just as in the living body a poison substance is counteracted by another poison.

The investigation of radiation effects on *Ascaris* eggs was undertaken by Fraülein Paula Hertwig in order to obtain definite evidence on the facts at the basis of the biological hypothesis. Her conclusion is that the division of the eggs is retarded, and pathological appearances are very soon noticeable after the radiation; chromatin is strongly affected, as already stated, although the centrosomes, spindles and other cell organs show no injury; unfavorable action of the cytoplasm is not to be assumed since no change can be seen. She is able to

find no ground for the lecithin theory, but interprets her facts from the contrary viewpoint. The effect of radium upon *Ascaris* eggs has been reinvestigated by Payne[10] who confirms the results just given.

Günther Hertwig, following the lead of his father, in his various studies set up four series of experiments, the A, B, C and D series. In the A series, eggs were radiated in the two-cell stage, or after fertilization; in the B series, sperm were radiated and then used to fertilize normal eggs; in the C series, eggs were radiated and then fertilized by normal sperm; in the D series, both eggs and sperm were radiated before fertilization. The experiments described in his first paper were on the frog's egg. He found that in the C series (normal sperm by radiated eggs) the injury increases with the duration of the radiation up to a maximum, and from there on decreases again as the radiation is prolonged. Only the radiated nuclei show the effects, and there is no evidence for the hypothesis that yolk which contains lecithin is being broken down. The injury is greatest on young tissue and on tissues which are to be highly specialized; it is productive of manifold disturbances of the developmental processes. In the frog development is possible with only a haploid nucleus, that is, only the half of the nucleus derived from one parent. Where the injury is severe to either the egg or sperm nucleus, the other is able to carry on the development, and in fact there is less interference with the regular course than in the case where both nuclei are injured slightly and both take part in the process, for here the injured half is no longer a factor; it is able only to stimulate development and then is eliminated from the process. In other words from the standpoint of heredity, this is a case of parthenogenesis. For the spermatozoon always has two functions to perform: it must initiate development, and it must carry the inheritance from the male line. In this case only the first function is accomplished, and parthenogenesis is the virtual result, the male pronucleus being eliminated from development by failing to unite with the female pronucleus. This result is important as evidence that the nucleus is the bearer of the inheritance substance and that in the beginning of development male and female nuclei are of equal significance.

Subsequently Günther Hertwig performed a similar set of experiments on the eggs of the sea urchin. Here he was able to work out the cytological details of the process and to establish firmly his view just stated, that the eggs after intense radiation really undergo parthenogenetic development. This is the most important point of his contribution, although he presents much evidence for the biological hypothesis.

A great many other experiments have been carried on in Hertwig's laboratory at Berlin, and all of them contribute to the same conclusions. The principles already discussed are the most important ones brought out, and they are supported by a large amount of evidence; many data also have been gathered from these experiments which are valuable from the standpoint of teratology. This, of course, is incidental to the evidence for the biological hypothesis.

In America, although much attention has been given to the medical aspects of radioactivity, very little work on the biological phases of the problem has been attempted. Bardeen[11] has carried out systematic ex-

[10] Payne, F., "A Study of the Effects of Radium upon the Eggs of *Ascaris megalocephala univalens*," *Roux Archiv.*, XXXVI., 1913.

[11] Bardeen, C. R., "Abnormal Development of Toad Ova Fertilized by Spermatozoa Exposed to Röntgen Rays," *Jour. Exp. Zool.*, IV., 1908. "Variations in Susceptibility of Amphibian Ova to the X-rays at Different Stages of Development," *Anat. Record*, III., 1909. "Further Stud-

periments of the toad's and frog's eggs, demonstrating that abnormal embryos result from radiation, and showing to a certain extent under what conditions they are produced. The kinds of abnormalities are described and experiments given to determine the periods of greatest susceptibility. The following are among his most important conclusions:

> These experiments show conclusively that both the male and the female sex-cells may be so altered by the X-rays as to give rise to the formation of monstrous forms. The susceptibility of the male and female sex-cells is approximately equal, although the abnormalities appear earlier in development and are greater when the ova are exposed. After fertilization until cleavage begins, the ova at first appear to be no more susceptible than the sex-cells before fertilization. During the earlier stages of cleavage the susceptibility of the eggs to the X-rays is markedly increased, but during the later stages of cleavage before closure of the blastopore the susceptibility of the eggs becomes much less, and after the blastopore is closed the power of the X-rays to influence development becomes strikingly reduced. The period of greatest susceptibility is the period during which there is the most rapid production of nuclear material.

Packard[12] has recently published an account of his experiments on the effect of radium on the fertilization of *Nereis*. These experiments were performed to ascertain how "early the development of the egg is affected by radium radiations when (1) the sperm is exposed; (2) when the egg is exposed; and (3) when the egg is exposed immediately after fertilization." His results are important, for he finds that, in addition to the usual effects such as retardation of development, multipolar spindles and the like, not only chromatin, but also the achromatic portions of the spindles and the cytoplasm, show the effects of the exposure. In eggs radiated before fertilization, it may happen that the alveolar layer of the cytoplasm is not extruded as is normally the case in the eggs of *Nereis*. If this occurs the maturation processes are much modified, resulting in diverse forms of chromosomes and spindles, with perhaps small asters scattered about through the cytoplasm; and various other irregularities may be present in the mitotic figures. Thus it will be seen that Packard's observations do not all agree with those made in Hertwig's laboratory. Parthenogenesis is not found to occur in *Nereis* as the result of exposure to radium, but it is a common observation that the eggs of that animal are not as favorable for parthenogenetic development as are those of the sea urchin upon which Hertwig worked.

For various reasons the hypotheses of both Hertwig and Schwarz are held to be insufficient to account for the phenomena observed by Packard, and he proposes another explanation, suggesting "that the radium radiations act indirectly on the chromatin and protoplasm by activating autolytic enzymes which bring about a degeneration of the complex proteids, and probably by affecting other protoplasmic processes in the same manner." This hypothesis is reached partly as a result of his own experiments and partly from a consideration of certain other work, and to some extent takes a middle ground between the other two, although in some phases it differs sharply from both. Cells contain a great many kinds of enzymes and it has been shown by a number of investigators that radium rays and X-rays have the property of modifying the action of some enzymes. Packard concludes that while many enzymes may be activated, "the lytic enzymes are more stimulated than those which play a synthesizing rôle." Where a

ies on the Variation in Susceptibility of Amphibian Ova to the X-rays at Different Stages of Development," *Amer. Jour. Anat.*, Vol. 11, 1911.

[12] Packard, Charles, "The Effect of Radium Radiations on the Fertilization of *Nereis*," *Jour. Exp. Zool.*, 16, 1914.

slight radiation results in acceleration, the synthetic processes may be supposed to be stimulated more than the destructive activities. This hypothesis is essentially chemical in nature and seeks to explain the morphological effects observed in the cells as the indirect result of enzymotic activity under the influence of the radiation.

Various new arguments for and against these hypotheses and theories have been brought out as new facts have developed. It is, therefore, necessary to consider the more important points critically.

The lecithin hypothesis was established on the basis of an experiment by Schwarz, showing that in the chicken egg yolk, containing lecithin, is broken down under the influence of radium. Against this hypothesis numerous objections are now to be raised. Hertwig pointed out that the decomposition was not determined by strict chemical methods, and Neuberg also criticizes it on chemical grounds, for lecithin is itself so unstable that only a very accurate chemical study could determine whether its decomposition was actually due to the radium radiation.

If the seat of the injury were in the yolk, little effect could follow radiation of the sperm before fertilization, for the sperm at most contains but little lecithin, and contrarily, much greater injury should result when the egg is radiated. As a matter of fact, there is very little difference between the results on the embryo, whether it is the egg or the sperm that is radiated before the fertilization. Egg nuclei are equally capable, with those of the sperm, of contributing the chromatin by which the parthenogenetic development previously described takes place, both at first are able to start the development of the egg so far as the hereditary units are concerned, and injuries arising from the disturbance of either are equally great.

Very little lecithin could be decomposed when so short a radiation of the sperm as a minute was employed, as by G. Hertwig; yet this radiation was sufficient to cause marked departures from the normal in the embryo. That no chemical poison is generated by lecithin decomposition is obvious, for in the case of the short radiation of the sperm the poison would be too dilute to cause effects equal to those resulting from longer radiation of the egg.

The lecithin theory takes no consideration of the fact that the most careful cytological study shows no morphological evidence of yolk destruction; nor of the fact that nuclei and especially chromatin do suffer marked changes as the results of the radiation. On the latter point all investigators agree, and it must be explained by any hypothesis which seeks to account for the changes produced. Furthermore, yet other cell constituents are acted on by radiation, for cell extracts and cell enzymes have been shown to be activated or retarded in their action, depending upon the conditions of the experiment.

In this connection, it is by no means clear how lecithin decomposition within the egg could prevent, as Packard found radiation to do, the extrusion of the alveolar layer of the cytoplasm, a phenomenon depending upon activities at the surface of the egg. And finally, it is not possible to account on this basis for the elimination of the sperm head from development, or for the fact that somatic nuclei of radiated *Triton* embryos contain only half or the reduced number of chromosomes.

Most of these arguments against the lecithin hypothesis were, of course, unknown at the time it was proposed. They are so much at variance with it, however, that it seems impossible to give it further serious consideration. It may be regarded as completely overthrown.

Similar explanations have been suggested by others, *e. g.*, by Hippel and Pagenstrecher[13] who find that *x*-radiation of rabbits produces an effect similar to that resulting from cholin injection and think that a toxin is developed which is transferred from mother to embryo, to the injury of the latter. The facts already brought out here show that this explanation is not a sufficient one, nor one of general application.

The objections to Hertwig's biological hypothesis are less serious than in the case just discussed, and they would scarcely hold against it in a less extreme form. It is, of course, true that the solution offered by Hertwig is incomplete in that it does not go far enough back into the organization of the cell; for, even on the assumption that the chromatin is the chief seat of injury, further explanation, which must ultimately be chemical in nature, is required to show how the injury is communicated to other parts of the cell and what the mechanism is by which its action is manifested.

According to the Hertwig hypothesis, chromatin above everything else in the cell suffers injury from radiation and the pathological conditions in the embryo are traceable to the injured chromatin, which may be regarded as a "contagium vivo" increasing, ferment-like, at each division.[14] Thus the mechanism of the cell provides the means for distributing the injury to each successive cell generation and for carrying it to all parts of the embryo.

To this theory, Packard has offered two criticisms. The concept, that the injured chromatin or a substance produced from it acts as a contagium vivo, is scarcely a solution of the problem, for it merely restates in another form certain facts observed and presents a picture of the problem itself from a different viewpoint without giving any explanation of the facts. It should again be pointed out that the ultimate solution must be chemical in nature. Packard also questions the assumption that the injurious substances developed in the nucleus must remain there and can not involve purely cytoplasmic structures even during division (for a normal haploid division of the egg chromosomes takes place if the radiated sperm head does not mechanically interfere in the spindle). A condition contrary to this assumption is given by Packard, showing that cytoplasmic structures are changed in *Nereis* eggs, for example in the case of eggs radiated before fertilization which fail to give off the alveolar layer and thus extrude the jelly as they should do. Obviously here the injury has been communicated to the egg cytoplasm, and is not in accord with the Hertwig assumption.

But the most serious objection to the Hertwig theory as it now stands lies in the fact that other than nuclear structures and substances are affected by X-rays and the radium rays. The failure to extrude the alveolar layer in *Nereis* eggs is a case in point, and in the same eggs abnormal spindles and asters occur as a consequence of radiation. It has been found by numerous investigators that radium rays have the power to affect enzymes, and the writer[15] has shown that X-rays are able to bring about modification in the activity of certain enzymes. Enzymes are derived from living tissues, and if it is possible to cause their modification outside of the cell by the use of radioactivity it is not improbable that they also undergo change while within the cells. In fact, the writer working with Miss Woodward[16] was able to prove that X-rays

[13] *Münchner Med. Wochenschr.*, No. 10, 1907.

[14] Hertwig, O., "Die Radiumkrankheit tierischer Keimzellen," *Arch. f. Mikr. Anat.*, Bd. 77.

[15] Richards, A., "The Effect of X-rays on the Action of Certain Enzymes," *Amer. Jour. Physiol.*, Vol. 35, 1914.

[16] Richards, A., and Woodward, A. E., "Note on the Effect of X-radiation on Fertilizin," *Biol. Bull.*, V., 28, 1915.

can be used to influence the activity of the cell extractive called fertilizin. This substance is extracted from sea-urchin and starfish eggs when the ripe eggs are allowed to stand in water for a short time, and it possesses the property of causing the agglutination of the sperm of its own species. Its behavior is in some respects comparable to that of an enzyme and it is possible that the substance contains enzymotic bodies. The experiments showed that radiation by X-rays is capable of changing the activity of fertilizin, and in general agrees with previous work that weak radiation is accelerative and strong inhibitive. Fertilizin is a substance derived from the living eggs and the extraction takes place while the egg is in the resting stage, sometimes even in the germinal vesicle stage; at this stage chromatin can scarcely play any part in the giving off of fertilizin. In this case, then, the radiation has had a considerable, and a measurable, effect on a cell substance independent of the chromatin or other nuclear structures. This fact can hardly be brought in line with the Hertwig hypothesis in its present form.

Yet it is true that chromatin and nuclear structures are greatly changed by radiation. In any true explanation that may be given this important fact must be dealt with. It is possible that a modification of the present form of the Hertwig theory in which the effect on enzymes is recognized may be sufficient to account for all the facts that are now known.

Packard has attempted such a modification in his suggestion "that the radium radiations act indirectly on the chromatin and protoplasm by activating autolytic enzymes which bring about a degeneration of the complex proteids, and probably by affecting other protoplasmic substances in the same manner." Against this hypothesis there is little that can be urged except the fact that it rests upon insecure evidence, there being but few actual observations or experiments which contribute to it. It is certain that radiation influences the activity of various enzymes, but there is very little evidence upon which to base the assumption necessary to the hypothesis that those enzymes which cause katabolic changes in the cell proteids are accelerated to a greater extent than those which have the opposite function. For this reason judgment can only be suspended until such a time shall come when accurate and more abundant data are at hand for attacking the problem.

Joly[17] has proposed a different kind of explanation to account for x-ray effects. Comparing the events which take place in a photographic film with those which occur in cells subjected to gamma or to x-rays, he supposes that the rays increase ionization in the tissue. The various results found are accounted for as due to differing degrees of ionization and to the presence or absence of an "intensifier" or an "inhibitor." No evidence from biological studies is given to support this hypothesis, however; it must, therefore, await experimental confirmation.

It will be seen from the foregoing review that all the investigations which have contributed to the development of these various theories either have had a morphological basis or were of a chemical nature. Along these lines there remains a great deal to be accomplished; we especially need more exact information on the nature of the injury which is done to the chromatin and to the cell organs.

But in addition to the morphological study the general problem must be studied by other methods. The question is raised as to what is the nature of the stimulus by

[17] Joly, J., "A Theory of the Action of Rays on Growing Cells," *Proc. Roy. Soc.*, Series B, Vol. 88, 1914.

which radioactivity affects organisms. According to Verworn a stimulus is "any change in the external agencies that act upon an organism." Are the rays of radium or X-rays comparable to the electric current, for example, in the manner in which they affect protoplasm? An experiment was performed by the writer to gain information on this point. A frog's leg was set up as a muscle-nerve preparation; when stimulated electrically it was found to react normally. The nerve, and later the muscle directly, were exposed to X-rays. When the brush discharge was carefully screened away from the preparation, the X-rays were unable to cause any contraction, even of the slightest extent, as shown on the drum of kymograph. This result was obtained repeatedly. While it gives no information as to real nature of the stimulus, it indicates that the stimulus of radioactivity is not comparable in its effects with that of the electric current.

Gager has adapted Verworn's biogen hypothesis to explain the manner in which radium rays act as a stimulus to organisms, and to provide the mechanism by which the stimulation may be supposed to operate. A stimulus is any change in the external agencies that act upon an organism. Metabolism according to Verworn "depends upon the continual destruction and continual reconstruction of a very labile chemical compound," biogen, which "develops at an intermediate point in metabolism, and by its construction and destruction comprehends the sum total of metabolism." It is not a protein nor living, for a molecule can not be alive. The ratio of construction and destruction of biogen molecules under normal conditions of equilibrium is $\frac{\text{construction}}{\text{destruction}} = 1$. Therefore, "the irritability of living substance depends upon the lability of the biogen molecules.

Now, Gager remarks:

> Both the dissimilatory and the assimilatory phases of metabolism may be stimulated. The degree of dissimilatory stimulation is, for equally intense stimuli, dependent upon the following factors:
>
> (*a*) The degree of lability of the biogen molecule.
> (*b*) The rapidity of the process of restitution after the functional destruction of the biogen.
> (*c*) The absolute number of biogen molecules present.
> (*d*) The conditions of the propagation of the stimulation.
>
> A dissimilatory stimulation, or depression, may be brought about by influencing any one of these individual factors. On the other hand, the degree of assimilatory irritability is dependent upon:
>
> (*a*) The quality of the raw material available for nutrition.
> (*b*) The means for working up the raw material into a suitable form of elaborated matter.
> (*c*) The quantity of suitable elaborated matter.
> (*d*) The rapidity of the transformation of the elaborated matter from the reserve depots into the biogen molecules.
>
> An assimilatory irritability or depression may arise through influencing each of these individual conditions.
>
> Radium rays, by acting on any one of the eight factors enumerated above, may, therefore, excite or depress processes of either assimilation or dissimilation.

Further, Gager points out the probability that radium rays may not affect their stimulation "by acting directly upon the biogen molecules, or whatever the reality may be that corresponds to this term, but by acting upon other substances in the individual cells, or by modifying some process either preceding or following the elaboration of the biogen molecule." The rays may produce their effect indirectly by acting upon some non-vital constituent other than the biogen, or upon some purely chemical process. Thus does he conceive the mechanism by which the rays produce the changes which they effect on organic bodies.

It will be seen that this elaborate conception of the method by which the results

are produced does not in any way conflict with the hypotheses already stated but is really an accessory to them. Although Gager probably had no such thought in mind, his conception is in complete accord with a theory of enzyme modifiability, which at the same time presents a picture of the manner in which the radiations affect protoplasm.

Early in the investigations the question arose as to whether the effects observed in the division of the egg might not be due to a change caused by the radiation in the permeability of the cell membranes to certain substances contained in the solution in which the eggs developed. It is known that surface changes due to the alteration of permeability account for many of the phenomena connected with the initiation of development and cell division, and by analogy it was argued that to similar changes might be due the retardation of division rate as well as other departures from normal as they occur in the radiated eggs. To test this question the writer undertook a series of experiments[18] in which several different tests for permeability change were used and all gave the same result: that the X-ray effects are not to be attributed to permeability changes caused by the radiation. In the first method, the larvæ of *Arenicola,* a marine worm, were employed, for it had been found that, when these larvæ are brought into any solution that causes permeability changes, a yellow pigment is exuded from the integument; no exudation could be observed under the influence of the radiation. The second method consisted of experiments conducted with the view to producing artificial parthenogenesis, for upon the basis of the current working hypothesis, artificial parthenogenesis is due to the cytolysis of the cortical layer of the protoplasm, which in its turn is correlated with permeability changes on the egg membrane; positive results from experiments to cause this phenomenon would therefore imply permeability changes. The experiments were unsuccessful in the attempt to cause parthenogenesis. Various modifications of the indicator method were used, all with the result that substances in solution were found to enter the cell, which had been stained with some neutral indicator, in this case neutral red, after exactly the same interval in both the radiated and the unradiated control cells. This shows that the radiation is ineffective in causing permeability changes in the cell membranes. These experiments warrant the conclusion that permeability changes are not the causal factors in the events which follow radiation.

From Gager's conclusions that radioactivity is a stimulus to metabolic processes, it may be inferred that the functions, as cell division, which even remotely depend on these processes would also be affected by radiation. Such an inference is borne out by the observations[19] made by the writer on the rate of division in *Planorbis* eggs that had been exposed to X-rays, for in these experiments it was found that a light radiation served to accelerate the first one or two mitotic cycles that followed it; after that injurious effects gradually asserted themselves. A strong radiation was directly inhibitive. The cytological study of the eggs used in these experiments has not been completed, so that it is as yet impossible to correlate the observations on the living eggs with changes in the finer details of their structure. It is of course possible that we have manifested in these physiological

[18] Richards, A., "Experiments on X-radiation as the Cause of Permeability Changes," *Amer. Jour. Physiol.,* Vol. 36, 1915.

[19] Richards, A., "The Effect of X-rays on the Rate of Cell Division in the Early Cleavage of *Planorbis*," *Biol. Bull.,* Vol. 27, 1914.

processes influences that make no impress on the morphological structure of the egg.

The case of the influence of X-rays on fertilizin, already referred to, provides another instance where the effects are without direct morphological representation. Doubtless others occur. These cases must of course be accounted for by any explanation of the effect of radiation on living organisms.

The facts, as they are at present known in regard to the effects of radioactivity on living matter, show that life processes are subject to marked changes under the influence of the radiation, a slight exposure being accelerative in most cases, while a more intense treatment is inhibitive or destructive. As a causal factor in these effects, the demonstrable injury to the chromatin of the cells is undoubtedly important; but there are also good evidences that the modifiability of enzymes under the action of the rays likewise plays a considerable part either directly or indirectly in the resulting injury.

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ARE RECESSIVE CHARACTERS DUE TO LOSS?

Since the presence-absence theory came into vogue it has become quite customary to regard recessive characters as due to the absence of something in the germ plasm on which the corresponding dominant character depends. The nomenclature of the presence-absence theory has been adopted by most writers on Mendelian inheritance, and it has afforded a useful and convenient method of expressing gametic formulas, although, as Morgan has shown, there are cases in which it leads to inconsistent results. While it is often recognized that this nomenclature is a purely symbolic scheme of indicating how certain characters behave in inheritance, the habitual employment of the system in the search for formulas which will designate by a series of large and small letters the gametic constitution of the organisms one is dealing with, has a strong tendency to influence one's views in regard to several important problems of heredity and evolution. I can not but think that the opinions of many students of genetics have been unduly influenced by their formulas. Formulas are excellent servants but bad masters. Almost involuntarily a certain interpretation is attached to their symbolism which is apt to have the practical effect of actual belief if it does not succeed in producing it.

Since the establishment of Mendel's law and its successful employment in elucidating many previously enigmatical phenomena of inheritance, heritable variations have commonly come to be considered as due to the addition or subtraction of discrete units of germ plasm, the bearers of unit characters. Professor Bateson in his "Problems of Genetics" says in regard to substantive variations that

> we are beginning to know in what such variations consist. These changes must occur either by the addition or loss of factors.

And further on he makes the following significant statement:

> Recognition of the distinction between dominant and recessive characters has, it must be conceded, created a very serious obstacle in the way of any rational and concrete theory of evolution. While variations of all kinds could be regarded as manifestations of some mysterious instability of organisms this difficulty did not occur to the minds of evolutionists. To most of those who have taken part in genetic analysis it has become a permanent and continual obsession. With regard to the origin of recessive variations, there is, as we have seen, no special difficulty. They are negative and are due to absences, but as soon as it is understood that dominants are caused by an addition we are completely at a loss to account for their origin, for we can not surmise any source from which they have been derived.

In his more recent address before the British Association, Bateson not only interprets all recessive characters as due to loss, but suggests that dominant characters may have arisen by the removal of inhibiting factors, thereby causing a "release" of the characters which previously lay latent in the germ plasm,